Analyzing Dextran in the Sugar Industry: A Review of Dextran in the Factory and a New Analytical Technique

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Introduction

The formation of dextran as a result of microbiological activity is well documented. Dextran is currently recognized as having significant financial impact in cane sugar more than in beet sugar as penalties are often levied by cane sugar refiners. The presence of dextran in the factory is known to cause multiple processing problems, each having financial impact beyond merely sucrose loss. The processing and financial impact of dextran on the total factory operation is significant. However, the ability to measure the presence of dextran may be a limiting factor in recognizing its true impact on sugar beet operations. It is known that losses in sucrose may occur in beets in storage even under favorable conditions. The adaptation of dextran testing in beet sugar processing can identify at any stage of the process where sucrose losses are occurring and to what extent. A recently developed test method using a monoclonal antibody procedure has just been introduced into the cane industry. The new test method eliminates the time consuming and labor intensive methods currently practiced for measuring haze formation. It also has the advantage of being able to test dextran in juice and syrup as well as on the final sugar, while the currently used methods test only the final sugar. Using the test in the factory will allow operations to be adjusted accordingly to prevent slowdowns and loss of sucrose. Comparison of test methods by procedure, precision and accuracy show that the monoclonal test procedure is clearly superior.

Dextran

Bacterial polysaccharides have been identified as a problem in sugar processing for more than 100 years. The polysaccharide most commonly associated with sugar processing problems is dextran. Dextran is a polyglucan characterized by a high percentage of \( \alpha-1,6 \) linkages. Dextran varies in size from very small (soluble) to very large (insoluble). It is the soluble dextrans that cause the greatest problems. They range in size from a few thousand to several million molecular weight. Dextrans are commonly produced in sugar process streams by bacteria of the genera *Lactobacillus*, *Leuconostoc* and *Streptococcus*. The most common species found in the sugar factory is *Leuconostoc mesenteroides*. Dextrans are formed by the breakdown of sucrose in a manner similar to enzymatic inversion, except the glucose portion of the sucrose molecule is linked into a growing molecular chain (dextran) rather than released.

Dextran can cause severe economic losses. Not only is sugar lost due to dextran formation but dextran itself causes processing problems. Increased juice viscosity, poor clarification, and crystal elongation are all associated with the presence of dextran. Dextran that enters the sugar production process remains with the process streams as shown below (Table 1).
Table 1 - Dextran in Process Streams Measured at Four Different Louisiana Sugar Factories

Dextran in syrup partitions into commercial sugar. At low dextran concentrations about 10% of the dextran in the syrup winds up in the sugar. It is believed that this percentage increases exponentially with higher dextran concentrations (Figure 1). There are observations that up to 30% of the dextran in syrup may wind up in the sugar when syrup dextran levels are greater than 5000 ppm/Brix.

![Figure 1 - Partition of Dextran from Syrup at a Commercial Sugar Factory](image)

The existence of quality points for dextran in raw sugar contracts has provided a standard for economic loss due to dextran. Dextran in raw sugar in excess of 250 MAU has been subject to a penalty by refiners. The penalty is calculated from a sliding scale based upon the sugar dextran concentration.

For example, the Number 14 Contract from Amstar Corporation (Table 2) calls for penalties on the value of the raw sugar as shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed Juice</td>
<td>2690</td>
<td>1094</td>
<td>1928</td>
<td>4650</td>
</tr>
<tr>
<td>Clarified Juice</td>
<td>2181</td>
<td>1094</td>
<td>1928</td>
<td>4560</td>
</tr>
<tr>
<td>Syrup</td>
<td>2602</td>
<td>1239</td>
<td>1986</td>
<td>3858</td>
</tr>
<tr>
<td>Dextran in Sugar (MAU)</td>
<td>Approximate ppm*</td>
<td>Penalty</td>
<td>Penalty @ $400/Ton</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------</td>
<td>---------</td>
<td>--------------------</td>
<td></td>
</tr>
<tr>
<td>250-350</td>
<td>558-710</td>
<td>0.007</td>
<td>$2.80/ton</td>
<td></td>
</tr>
<tr>
<td>350-450</td>
<td>710-862</td>
<td>0.009</td>
<td>$3.60/ton</td>
<td></td>
</tr>
<tr>
<td>450-550</td>
<td>862-1014</td>
<td>0.011</td>
<td>$4.40/ton</td>
<td></td>
</tr>
<tr>
<td>&gt; 550</td>
<td>&gt;1014</td>
<td>0.013</td>
<td>$5.20/ton</td>
<td></td>
</tr>
</tbody>
</table>

* There is a correlation between MAU and ppm based on T-40 (40,000 molecular weight) dextran:

$$ ppm = \frac{(MAU + 118)}{0.659} $$

and conversely,

$$ MAU = \frac{(ppm \times 0.659) - 118}{1} $$

Table 2 - Amstar Number 14 Sugar Contract Dextran Penalty Example

Factory operations have been geared to minimize this loss by blending high and low dextran sugars. This requires adequate warehouse space and is not always a solution for factories, which have limited storage or a shortage of low dextran sugar. Losses due to penalty also do not account for the real costs of dextran caused sugar loss and operational problems.

Dextran in the Factory

The amount of dextran entering a cane sugar factory is, of course, a function of the location, weather, condition of the crop, etc. The level of dextran in factory mixed juice is the sum of dextran from cane after harvesting; dextran formed between harvest and grinding (including shipping time and time waiting in the yard); and dextran formed during the milling process. A five year statistical study of the Louisiana sugar industry, with whole stalk harvesting, showed that the majority of the time this industry had less than 250 ppm of dextran in their juice. But a significant portion of the time the juice contained more than 250 ppm dextran, even though the dextran levels are not high enough to cause noticeable operational problems (Figure 2).

![Figure 2 - Dextran in Juice in Louisiana Sugar Factories](image-url)
It is well established that when dextran is high, boiling house performance drops. Dextran levels as high as 10,000 ppm/Brix have been reported in syrups. These changes have been attributed to dextran-mediated viscosity alterations, changes in heat transfer characteristics and changes in the crystallization characteristics of the sucrose. The most damaging effects of high levels of dextran are seen on crystal growth. Sucrose crystallizes as elongated crystals in the presence of dextran. Commercially this results in loss of recoverable sugar to molasses. Laboratory studies in Australia have shown a sugar loss to molasses of between 1.2 and 1.4 purity points can be expected for every 1,000 ppm/Brix of dextran in molasses. This is equivalent to about 250 ppm/Brix in mixed juice.

**Dextran Effect on Sucrose Loss at the Centrifugals**

Dextran content in massecuite has a certain influence on both sugar crystallization and centrifugation processes. Studies helped demonstrate the correlation between dextran content and the separation process parameters. During the studies, dextran content ranged from 22-905 MAU. It was noticed that higher levels of dextran corresponded to lower purity of massecuite. Also, the amount of wash time on the centrifugal required to get the required quality of sugar (98.6% Pol and <2500 color) was increasing as the dextran content was rising. Total centrifugal cycle time had to be increased also with the rise in dextran levels. Purity loss due to dextran formation can be 0.4-0.5 percent sucrose, making purity of massecuite lower, and slowing down crystal growth rate. All of these factors lead to less uniform crystal sizes, increase the amount of fine crystals, and give a higher coefficient of variation. Together with higher viscosity this significantly decreases purging capability of green syrup. It is clear that the massecuite quality significantly affects the sugar crystal yield making it to be 96.5% for 22 MAU dextran with 85.5% purity massecuite, and only 86% for 380 MAU dextran and 84.3% purity massecuite. This can be explained by the better purgability of the higher quality massecuite that helps reach a thicker cake in the centrifugal and by a smaller amount of water dissolving less sugar.

**Dextran Effect on Sucrose Loss in Molasses**

Factory studies in Louisiana have shown losses to molasses of between 0.55 and 0.625 purity points for every 1,000 ppm/Brix in molasses. The difference is believed to be due to the greater efficiency in crystallization of commercial compared to laboratory crystallizers. The amount of dextran in molasses obviously varies, however, in Louisiana, under good harvest conditions dextran in final molasses averages around 2,900 ppm/Brix. This would mean an average loss of 1.6 to 1.8 pounds of sugar per ton of cane. Sugar losses weighted for dextran distribution show that in Louisiana an average of 3 pounds of recoverable sugar/ton of cane is lost to molasses due to dextran (Table 3).

<table>
<thead>
<tr>
<th>Sugar Losses</th>
<th>Lbs/Ton</th>
<th>$ per Day Loss*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar converted to dextran prior to processing</td>
<td>0.72</td>
<td>$1,440</td>
</tr>
<tr>
<td>Sugar loss to molasses due to dextran</td>
<td>3.01</td>
<td>$6,020</td>
</tr>
<tr>
<td>Sugar loss due to dextran</td>
<td>3.73</td>
<td>$7,460</td>
</tr>
<tr>
<td>TOTAL DAILY LOSS</td>
<td>7.46</td>
<td>$14,920</td>
</tr>
</tbody>
</table>

* Cost of lost sugar at $0.20/Lb. at a 10,000 ton per day sugar factory.
(Based on a stoichiometry that 3 pounds of sucrose are used to make 1 pound of dextran.)

Table 3 - Direct Cost of Sugar Loss due to Dextran
Minimizing the Effects of Dextran on Sugar Production

What can a sugar factory do to minimize sugar loss to dextran? Sugar losses in the field can only be addressed through a properly managed harvest program, which minimizes delay between cut and crush. In the factory, juices, syrups, massecuites and molasses must be managed to minimize viscosity effects. Dextran in solution increases viscosity, decreases clarification efficiency, lowers evaporation rates and reduces heat transfer. It will slow both boiling times and purging of centrifugals, leading to a decrease in factory capacity.

Sugar production is a continuous process and every stage in the process can adversely affect those further down the line. Losses can only be minimized by maximizing the efficiency at each stage. For dextran, this means taking steps to reduce the viscosity of the product flow throughout the factory.

Benefits of Removing Dextran

The best solution for dextran, short of not having any, would be the ability to remove the polymer in much the same way α-amylase is used to remove starch. The enzyme dextranase has been shown to be effective in treating dextran problems at sugar factories\textsuperscript{17,18}. With dextranase use, elongation of grain ceases, boiling times are shortened, product flows more smoothly through the boiling house, and sugar filterability improves. Sugar that is normally lost to the molasses may be recovered.

Testing for Dextran in the Factory

In factories where good microbiological control exists, the sole source of dextran is that in the cane when it arrives from the field. Testing should be conducted not only on the sugars produced, but also on the juice entering the process. This may include testing trucks during core sampling to isolate farmers who deliver poor quality beets or cane. Process streams such as clarified juice, syrups, remelts and C-sugar should be monitored for dextran to pinpoint locations of contamination and to avoid mixing high dextran materials with low dextran streams. Sugar should be routinely monitored so high dextran sugars can be blended with low dextran sugars to minimize any penalties.

Analytical Methods for Dextran

The problems encountered in designing a procedure for use in sugar processing to detect dextran are many fold. Chemically, the problem is detection of a small amount of a specific polysaccharide in the presence of large amounts of other polysaccharides and sugar. Complicating the problem is the fact that dextran is not a uniform molecule, but varies both in size and structure. Underlying this is the industry need for a simple, rapid and repeatable assay, which can be used throughout the factory and not be limited only to the final sugar. Since even small amounts of dextran can affect sugar production, testing is desirable across the production process. Currently, only raw sugar is routinely monitored for dextran at the factory or the refinery.

The Haze method\textsuperscript{19} is still the standard method for levying dextran penalties on raw sugar. Continuous research on methods for analysis of dextran has produced numerous other analytical
approaches, ranging from alcohol precipitation to enzyme electrodes. Each method proposed for analyzing dextran is based upon different physical or chemical principles and each has some disadvantages. From the view of the sugar analyst the following five criteria (Table 4) are good indicators of the suitability of a given dextran analytical procedure. All currently published methods fail in one way or another against these criteria.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplicity</td>
<td>The assay should not require a high degree of skill in the actual manipulation of the test or a high degree of skill in maintaining the instrumentation.</td>
</tr>
<tr>
<td>Speed</td>
<td>The test should be capable of being conducted rapidly so that decisions can be made on process modifications in response to test results.</td>
</tr>
<tr>
<td>Precision</td>
<td>Precision or repeatability is more important than absolute accuracy (although a consistent degree of accuracy is necessary) particularly if the analytical results are going to impact financial concerns such as penalties.</td>
</tr>
<tr>
<td>Range</td>
<td>The test system should be capable of handling a wide range of analytical samples with little or no modification.</td>
</tr>
<tr>
<td>Availability</td>
<td>The test must be based on readily available equipment and reagents.</td>
</tr>
</tbody>
</table>

Table 4 - Criteria for an Acceptable Analytical Procedure

Most research has concentrated on analysis of dextran in raw sugar. This happens to ease the analytical problem, as sugar crystallization separates dextran from the majority of interfering polysaccharides. If not used on sugar, a dextran separation step is usually part of the analysis. This step can add significant time to any procedure. The various analytical procedures can be classified by the method of separation they use to separate dextran from the sample prior to quantification (Table 5).

<table>
<thead>
<tr>
<th>Analytical Method</th>
<th>Separation Process *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haze Method</td>
<td>Crystallization</td>
</tr>
<tr>
<td>Roberts Method</td>
<td>Alcohol Precipitation</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>None</td>
</tr>
<tr>
<td>Immunochemical</td>
<td>None</td>
</tr>
</tbody>
</table>

Table 5 - Dextran Analytical Methods Classified by Separation Process

(*Separation of dextran from the sugar)

Instrument and immunochemical methods are generally the only procedures, which do not require pre-separation of the dextran from the sample.
A comparison of the Haze Method, an alcohol-based test (Roberts\textsuperscript{21}), an instrumental method (Gel Permeation Chromatography, GPC) and two immunochemical tests illustrates the problems. The Haze Method is one of the simplest of the current methods for dextran analysis. It is reasonably rapid and the materials for conducting the test are readily available. However, its range is limited to sugars. The precision of the test is good but the accuracy of this method is poor. The Haze Method loses sensitivity when the size of the dextran detected is less than 150,000 mw. It also overestimates the amount of dextran present when the dextran concentrations are high (Figure 3).

![Dextran Measured by Haze Test](image)

**Figure 3 - Dextran Measured by Haze Method**

The ability of the Roberts method to detect low molecular weight dextrans is better than that of the Haze Method\textsuperscript{22}. However, it has been reported that both the Haze and the Roberts test are non-specific for dextran\textsuperscript{23}. The Roberts test is also limited in range to sugars. Its sensitivity is better than that of the Haze Method as it solved the variable molecular weight problem and the materials for the procedure are readily available. It is not as rapid as the Haze Method and requires better analytical skills.

Gel Permeation Chromatography (GPC) fails in the areas of simplicity and speed. Its range is good, being adaptable to all sugar process streams. Immunological methods are both simple and rapid. In fact, immunological methods fit all the criteria except availability. They require specialty reagents, which are not readily available. All methods of analysis tested were internally consistent, but they do not agree well among each other.
Variability in Dextran Analysis

If a series of raw sugars are assayed for dextran concentration by different methods, each method gives a different result (Table 5).

<table>
<thead>
<tr>
<th>Sugar Sample</th>
<th>Haze*</th>
<th>GPC</th>
<th>Monoclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>167</td>
<td>243</td>
<td>248</td>
</tr>
<tr>
<td>2</td>
<td>384</td>
<td>531</td>
<td>445</td>
</tr>
<tr>
<td>3</td>
<td>384</td>
<td>413</td>
<td>458</td>
</tr>
<tr>
<td>4</td>
<td>481</td>
<td>557</td>
<td>615</td>
</tr>
<tr>
<td>5</td>
<td>409</td>
<td>623</td>
<td>244</td>
</tr>
<tr>
<td>6</td>
<td>269</td>
<td>364</td>
<td>285</td>
</tr>
<tr>
<td>7</td>
<td>418</td>
<td>664</td>
<td>284</td>
</tr>
<tr>
<td>8</td>
<td>512</td>
<td>546</td>
<td>485</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>374</td>
<td>197</td>
</tr>
<tr>
<td>10</td>
<td>409</td>
<td>610</td>
<td>379</td>
</tr>
</tbody>
</table>

* MAU converted to ppm from a calibration curve, results reported as ppm.

Table 5 - Dextran (ppm) in 10 Different Raw Sugars as Measured by Different Methods

The non-specificity of the various methods shown in Table 5 is quite apparent and demonstrates that the various methods do not correlate very well. The Haze Method produces turbidity which is non-specific and does not detect low molecular weight dextran. The GPC Method often detects compounds other than dextran. The monoclonal antibody is specific depending upon the antigen used for antibody production and it detects a wider range of molecular weights. It is difficult to compare analytical methods that vary in their principle of detection for naturally occurring dextran having various molecular weights and branching properties.

A summary of the utility of current methods for monitoring dextran during sugar processing shows that of all the assay methods, only the immunochromatography (antibody) approach meets the set of defined criteria (Table 6).

<table>
<thead>
<tr>
<th>Method</th>
<th>Simplicity</th>
<th>Speed</th>
<th>Precision</th>
<th>Range</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haze</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Roberts</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GPC</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Antibody</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
</tbody>
</table>

Legend: + = Good; - = Poor; * = New

Table 6 - Comparison of Test Methods to Defined Criteria for Dextran Analysis

Immunological (antibody) methods have the most promise of meeting the above criteria for a useful assay. There are at least two reported methods for immunodextran analysis in sugar process streams, that of Curtin and that of Clarke. In both reports dextran is quantified by measuring...
formation of antibody complexes with a nephelometer. The antibodies for either test have not been readily available in the past, but a monoclonal antibody is now commercially available to the sugar industry.

**Basis for Immunological (Antibody) Assay Methods**

Antibodies are specific glycoproteins complimentary to specific foreign molecules (antigens or immunogens) produced in the blood stream of animals. Antibodies bind specifically to the immuogen, which initiated their production. However, only specific localized areas on the molecular surface of the immunogen, called an epitope, reacts to trigger the antibody production. There may be many epitopes for any given immunogen. A specific antibody to a single epitope is produced by a single clone of plasma cells. Such a single specific antibody is called a *monoclonal antibody*. Because each antigen can have many epitopes, the whole serum derived from stimulus by a single antigen is called a *polyclonal antiserum*.

The ease of production as well as the heterogeneity of structures makes a polyclonal sera valuable in certain diagnostic applications. However, most applications are better served by a reliable source of specific monoclonal antibody. This is virtually impossible to achieve with normal plasma cells as they die after a few divisions. Monoclonal antibodies are produced naturally in diseases such as multiple myeloma, where a single plasma cell becomes malignant and multiplies uncontrollably. It is now possible to fuse myeloma cells with plasma cells to produce a hybrid cell, called a *hybridoma*, which has the capacity of producing a single, monoclonal, antibody as well as the capacity for unlimited division. This has allowed the large scale production of single, specific antibodies. The following illustrates the steps necessary to produce a monoclonal antibody for dextran detection.

1. Elicit antibody response.

   ![Diagram](image)

2. Isolate spleen cells and fuse with myeloma cells.

   ![Diagram](image)

3. Screen and isolate desired hybridoma.

   ![Diagram](image)

4. Production of monoclonal antibody.

   ![Diagram](image)
Hybridoma cells can be cultured by one of two general techniques. The simplest is by formation of a soluble tumor or ascites in the peritoneum of a mouse. The tumor is injected with the hybridoma where it forms a myeloma tumor, which produces high concentrations of monoclonal antibody in the mouse. There is, however, a limit to the amount of material that can be produced from a single animal. The second method is to culture the cells in vitro in fermentors or bioreactors. The cell culture fluid will contain the monoclonal antibody. In vitro is the most reliable method for producing monoclonal antibody for use in the sugar industry.

**Conclusion**

The literature refers to the presence of dextran in the beet industry and the fact that it causes process related problems. Dextran is formed in appreciable amounts in beets in storage under unfavorable conditions, and can constitute a real problem. Dextran can partially seal filter cloths, and have seriously impeded rotary vacuum filtration of first carbonation sludge in a number of instances. Dextran appreciably affects the polarization of the process juices. Its presence in appreciable quantities can cause error in the measurement of the sugar entering the factory. In the presence of dextran, the crystal habit may become distorted along another axis to form elongated, needle crystals. The bulk density of such crystals may be reduced to as low as 48 pounds per cubic foot.

The new monoclonal antibody test method is more sensitive and allows testing of various process streams. Of greatest importance, the test can be conducted in only 3-5 minutes, a significant improvement over all other methods. The antibody attaches to dextran and produces a haze. The amount of haze formed is directly proportional to the amount of dextran present. A specially modified nephelometer (turbidimeter) is used to measure the turbidity of the haze formed by the reaction of the antibody with dextran. Monoclonal antibody is supplied freeze-dried in a sealed vial. The result may be expressed as ppm in solution or as ppm on solids. The test is also portable, allowing on-site testing at suspected trouble areas with immediate results.

The Cane Sugar Industry has examined the impact of dextran on the economics of processing as presented in this paper. The Beet Sugar Industry would likely benefit from doing the same. The availability of the new analytical method (monoclonal antibody) for rapidly testing dextran in the factory will make this investigation easier to accomplish.
References


